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# Cytotaxonomic Study of Ferns from China I.
# Species of Yunnan

Su-Juan LIN[a], Kunio IWATSUKI[a] and Masahiro KATO[b]

[a]Faculty of Science, Rikkyo University, 3-34-1 Nishi-ikebukuro, Toshima-ku, Tokyo, 171 JAPAN;
[b]Department of Biological Sciences, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Tokyo, 113 JAPAN

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Sporophytes of 18 species belonging to 15 genera of ferns were grown up from the spores collected in Yunnan, Southwest China and cultured in the Botanical Gardens, University of Tokyo. The chromosome numbers were observed from the root tips of these young sporophytes. The putative reproductive manners of most species were inferred from cytological characters and spore counting. Eleven new records (including four cytotypes) were given. *Hypolepis punctata* 2n=116 (4x) has the basic number of x=29, different from the previous reports x=49, 52, and 98. *Pteris dactylina* is an agamosporous triploid. *Gymnopteris vestita* has the basic number of x=29 but not 30; *Thelypteris* sp., *Deparia japonica* and *D. petersenii* are sexual hexaploids; *Adiantum philippense* has somatic chromosome number of 2n=60 (2x), and 32(16) spores in each sporangium. It may be an agamosporous diploid. *Lepisorus scolopendrium*, which has sometimes been treated as a variety of *L. excavatus* (n=35), was determined here to be 2n=100. The cytological data support *L. scolopendrium* to be separated from *L. excavatus*. Some problematic species were discussed from cytotaxonomical points of view.

Yunnan is a province situated in southwestern China. This province is noted for its very rich pteridophyte flora. There are about 1285 species recorded in Yunnan (Cheng 1989) and are counted as nearly a half of 2600 in whole China. In analyzing biological aspects of flora and biogeography of Yunnan ferns, the informations on basic chromosome number, ploidy, and reproductive system are very important. Cytological studies have been made by various researchers, and about 300 Chinese taxa of the pteridophytes have been observed and reported by Roy and Holttum (1965), Chiu (1981), Wang (1981, 1985, 1987, 1989a, b), Wang and Zhang (1981), Wang and Sun (1982), Wang and Xia (1984), Wang et al. (1984), Weng (1985a, 1985b, 1989a, 1989b, 1990), Weng and Qiu (1988), Li (1988), Kato et al. (1992), and Nakato et al. (1995). Among them, Kato et al. (1992) reported chromosome numbers and reproductive manners of 90 fern species from Yunnan. However, much more informations are expected for studying evolution, diversity, and biogeography of Yunnan ferns.

We collected the herbarium specimens and mature spores in the fields and young sporophytes grown up from the spores for cytological investigation. In the present paper, the cytotaxonomy of 18 species from Yunnan is reported, some of the counts being made for the first time.

## Materials and Methods

Viable spores were collected in 1990 in Xishuangbanna and Dali Prefecture, Yunnan (Lin et al. 1992; Table 1) and young sporophytes for cytological studies have been raised up. Voucher specimens are deposited both in the herbarium, Kunming Institute of Botany, Academia Sinica (KUN) and the herbarium, Botanical Gardens, Faculty of Science, University of Tokyo (TI).

The methods of cultivation of spores and sporophytes are the same as described in Lin et al. (1992). For observing somatic chromosome numbers, fresh root tips of the young sporophyte were pretreated with 0.002 M 8-hydroxyquinoline solution for 3 hours at 18–20°C, fixed in 45% acetic acid solution for 10 minutes macerated in mixed solution of 1N HCl and 45% acetic acid (3:1) for 1 minute at 60°C, stained with 2% aceto-orcein, and then squashed. For avoiding the mis-counting by mixed-in of spores of other species, 3–5 individuals were observed in each species (in each pot of cultivation). For comparison, meiotic chromosomes were counted in a sample of *L. ensifolia* by Manton's (1950) method.

## Results and Discussion

The results are shown in Table 1 and Figures 1–17. Eighteen species belonging to 15 genera in 11 families were examined cytologically. Seven species and 4 cytotypes are new records in China (in Table 1, one asterisk denotes new records, two asterisks new record for Chinese plant of the species; no asterisk means that the record agrees with existing records of the chromosome information of the species concerned.

1. *Angiopteris* sp.

*Angiopteris* is an eusporangiate genus belonging

Table 1. Materials and chromosome numbers of species from Yunnan

| Family/Genus/ Species | Chromosome number (ploidy level) | Spore number | Locality and voucher |
|---|---|---|---|
| Angiopteridaceae | | | |
| *Angiopteris* x=40 | | | |
| *A. sp. | 2n=c.120 (3x) | | Menlun, Xishuangbanna; c.900m alt.; 725; Spo. 113 |
| Lindsaeaceae | | | |
| *Lindsaea* x=34, 40, 44, 47, 50 | | | |
| ***L. ensifolia* Sw. | 2n=c.130 (3x) | 32 | Menlun, Xishuangbanna, c.1100m alt.; 797; Spo. 121 |
| | n=88 (4x) | 32 | Menlun, Xishuangbanna, c.900m alt.; 1045 |
| Hypolepidaceae | | | |
| *Hypolepis* x=29, 39, 45, 49, 50, 52 | | | |
| **H. punctata* (Thunb.) Mett. | 2n=116 (4x) | 64 | Menlun, Xishuangbanna, c.900m alt.; 710; Spo. 111 |
| Pteridaceae | | | |
| *Pteris* x=29 | | | |
| *P. cretica* L. | 2n=58 (2x) | 32 | Qinbixi, Dali; Spo. 56 |
| **P. daclylina* Hook | 2n=87, 88 (3x) | 32 | Qinbixi, Dali; Spo. 75 |
| Sinopteridaceae | | | |
| *Onychium* x=29 | | | |
| *O. japonicum* (Thunb.) Kunze | 2n=116 (4x) | 64 | Qiliqiao, Dali; Spo. 8 |
| *O. lucidum* (D. Don) Spr. | 2n=116 (4x) | 64 | Pingpo, Dali; Spo. 35 |
| *Pellaea* x=29, 30 | | | |
| ***P. nitidula* (Wall.) Bak. | 2n=116 (4x) | 64 | Qiliqiao, Dali; Spo. 11 |
| Adiantaceae | | | |
| *Adiantum* x=30 | | | |
| ***A. philippense* Linn. | 2n=60 (2x) | 32 (16) | Menlun, Xishuangbanna; Spo. 89 |

Table 1. Continued

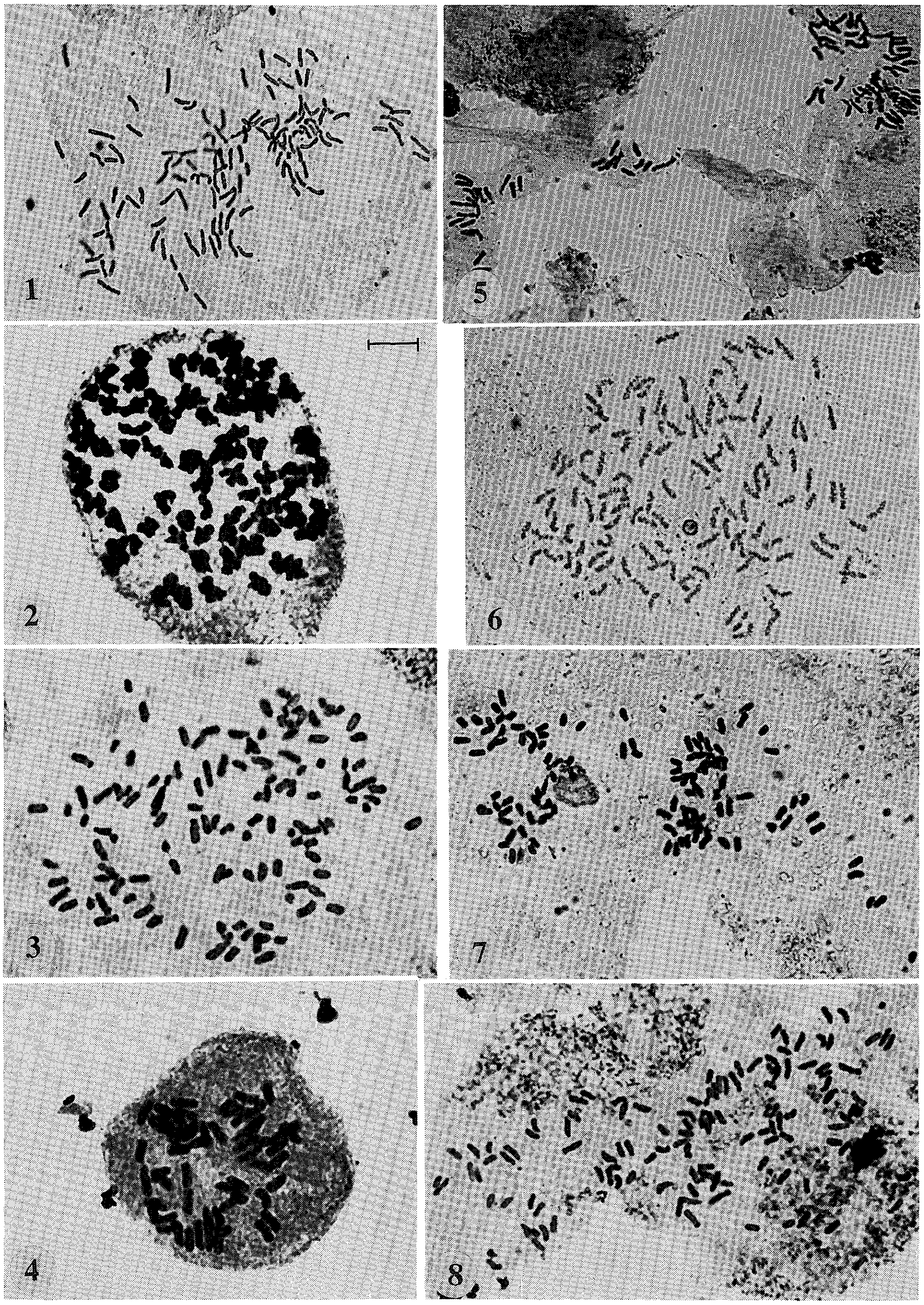
| Family/Genus/ Species | Chromosome number (ploidy level) | Spore number | Locality and voucher |
|---|---|---|---|
| Hemionitidaceae | | | |
| *Gymnopteris* x=30 | | | |
| ***G. vestita* (Wall.) Underw. | 2n=58 (2x) | 64 | Qinbixi, Dali; 78; Spo. 74 |
| Thelypteridaceae | | | |
| *Parathelypteris* x=31 | | | |
| *P. beddomei* (Bak.) Ching | 2n=62 (2x) | 64 | Zhonghefeng temple, Dali; 2450; Spo. 31 |
| *Pseudophegopteris* x=31 | | | |
| *P. levingei* (Clarke) Ching | 2n=62 (2x) | 64 | Qinbixi, Dali; Spo. 60 |
| *Pronephrium* x=36 | | | |
| **P. gymnopteridifrons* (Hayata) Holttum | 2n=72 (2x) | 64 | Menlun, Xishuangbanna, c.900m alt.; 716; Spo. 109 |
| *Thelypteris* x=27, 29, 31, 36 | | | |
| *T. aurita* (Hook.) Ching | 2n=c.178–180 | 64 | Qiliqiao, Dali; Spo. 5 |
| Blechnaceae | | | |
| *Woodwardia* x=17, 34 | | | |
| *W. radicans* Sm. | 2n=68 (2x ?) | 64 | Menlun, Xishuangbanna, c.900m alt.; Spo. 114; 726 |
| Woodsiaceae | | | |
| *Deparia* x=40, 41 | | | |
| ***D. japonica* (Thunb.) Kato | 2n=c.240 (6x) | 64 | Qiliqiao, Dali; Spo. 13 |
| ***D. petersenii* (Kunze) Kato | 2n=c.240 (6x) | 64 | Qiliqiao, Dali; Spo. 10 |
| Polypodiaceae | | | |
| *Lepisorus* x=35, 50 | | | |
| **L. scolopendrium* (Ham. ex D. Don) Mehra & Bir. | 2n=100 (2x) | 64 | Zhonghefeng temple, Dali; 2380; Spo. 26 |

to the Marattiaceae. It includes about 200 species centering in the tropics of Southeast Asia (Kramer and Green 1990, Wu and Ching 1991). This is a taxonomically problematic genus for its poor characters in classification and thus caused an ambiguous definition of every species (Iwatsuki 1995). One of the three Japanese species and 22 among 60 Chinese species were already observed cytologically by Nakato (1988, 1996), Li (1989) and Tsai and Shieh (1983). All the species ever observed were diploid, 2n=80 or n=40, and no contributive information was available for taxonomy. In this study, however a sample from Menlun, Xishuangbanna (Table 1), was counted as somatic chromosomes 2n=c.120 (Fig. 1), a triploid. The observation of the meiotic chromosome is needed to determine the reproductive manner. In spite of this incomplete stage of data, it suggests that polyploids are existing in this group and probably played an important role in the differentiation in this group of ferns.

2. *Lindsaea ensifolia*

This is one of the common and polymorphic species in the genus *Lindsaea*. A number of infraspecies forms within this variable species are known to distribute in different areas, and intermediates among them of intraspecies were reported (Kramer 1971). The chromosome numbers n=88 (4x) and n=44 or 45 (2x) were recorded in *L. ensifolia* by Manton and Sledge (1954). The basic chromosome number was regarded as x=44.

In the present study, a sample fixed in a field of Xishuangbanna (Table 1) was observed as n=88 (Fig.



Figs. 1–8. Photomicrographs of chromosomes. 1. *Angiopteris* sp., 2n=120. 2. *Lindsaea ensifolia*, n=88. 3. *Hypolepis punctata*, 2n=116. 4. *Pteris cretica*, 2n=58. 5. *P. dactylina*, 2n=87. 6. *Onychium japonicum*, 2n=116. 7. *O. lucidum*, 2n=116. 8. *Pellaea nitidum*, 2n=116. Scale bar = 10 μm.

2) for chromosomes and 32 spores in each sporangium. According to the 32-spored sexual reproductive mode of the Lindsaeoid ferns (Lin et al. 1990), this individual is considered to be a sexual tetraploid. Another sample, whose sporophytes were grown up from the spores collected in Xishuangbanna in the site near the former sample (Table 1), was examined to have 2n=c.130 (3x) chromosomes and its parent is 32-spored. In form and size of the spores of the parent appear either normal or subnormal. It is possible that this individual is an agamosporous triploid. To have further evidences, however, it is expected to observe meiotic chromosomes and sporogenetic process.

As there are both the triploid and the tetraploid exist in Xishuangbanna, it is suggested that a diploid form of *L. ensifolia* in highly probable distributes in Xishuangbanna, Yunnan, too; the variations of this species complex are in many cases concerned to their variable cytotypes.

3. *Hypolepis punctata*

*Hypolepis* is a tropical and extratropical genus of 50 species including 6 in China (Wu and Ching 1991). There is a confusing array of chromosome numbers with n=29, 39, 51–53, c.92, 98, and 2n=c.92, 104, c.150, 208 etc. in this genus. The base number 29 was conjectured to be primitive, and the number n=98 from Japan (Kurita 1967, 1972, Mitui 1976) was inferred to be an aneuploidy (Brownsey 1983, Kramer and Green 1990). *Hypolepis punctata* is an ill-defined species, which was considered by Brownsey (1983) to form as many as four different species from Japan, northern India, Taiwan, Ceylon and Malaya. The various chromosome numbers were recorded as n=52, 51–53, 92, 98, 100, 104 (Manton and Sledge 1954, Mehra and Khanna 1959, Kurita 1962, 1967, 1972, Mitui 1968, 1975, 1976, Tsai 1973, Ghatak 1977, Bir and Vasueleva 1978, Wang et al. 1984).

In our observation on Yunnan plants, *H. punctata* has somatic chromosomes 2n=116 (Fig. 3) and its parent is 64-spored. The Yunnan form is a sexual tetraploid with the base number x=29, and this is a new record for the base number of this species. In such a species complex, speciation and resulted species diversity seem to concern with a variety of cytological variation, including polyploidy, polybasic chromosome number, and aneuploidy.

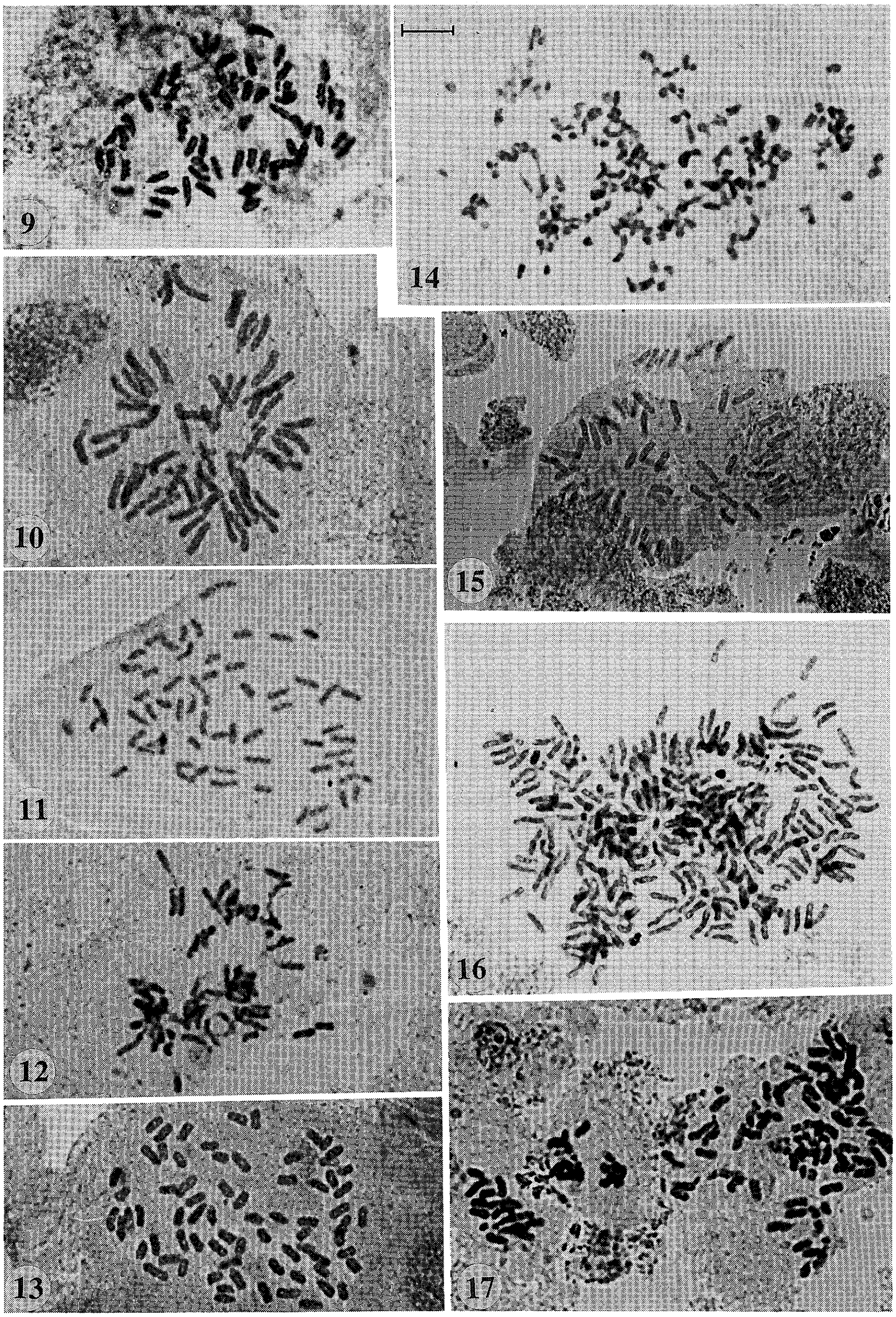
4. *Pteris dactylina*

*Pteris* is fairly well known in cytological point of view. Polyploidy, agamospory, and hybridization have important roles in speciation in this genus. *P. dactylina* is a common species in Southwest China and Himalaya. It was considered to relate to a sexual diploid species *P. henryi* Christ (Shing et al. 1990). *Pteris dactylina* was observed here cytologically from the first time. It is an agamosporous triploid species with the chromosome number of 2n=87 or 88 (Fig. 5).

5. Sexual tetraploid species of *Onychium* and *Pellaea*

*Onychium* Kaulfuss is a world-wide genus, with perhaps 8 species. Of which *O. japonicum* is a variable polyploid complex with diploid, triploid, tetraploid, pentaploid, and haxaploid (Roy et al. 1971, Bir 1973, Weng 1985, Kato et al. 1992). The chromosome base number is x=29. *Onychium lucidum* has been treated as a variety of *O. japonicum* by several authors (Ching 1934, Shing et al. 1990). It is characterized by larger size of plants, stouter habit, and coaser texture with thick stipes shaded rufo-brown. It was reported as triploid agamosporous (Roy et al. 1971). The sexual tetraploid of *O. lucidum* with somatic chromosomes of 2n=116 is a cytotype newly recorded to China (Fig. 7). The taxa with both sexual and agamosporous reproductive manners are of interest for their genetic structure of species.

*Pellaea nitidum* was reported as n=29 (2x) and n=58 (4x) in India (Bir and Rai 1984, Verma and Goloknath 1967). A specimen examined here has a somatic chromosome number 2n=116 (Fig. 8) and its parent is 64-spored, and this is a sexual tetraploid, a record new to China.



Figs. 9–17. Photomicrographs of chromosomes. 9. *Adiantum philippense*, 2n=60. 10. *Gymnopteris vestita*, 2n=58. 11. *Parathelypteris beddomei*, 2n=62. 12. *Pseudophegopteris levingei*, 2n=62. 13. *Pronephrium gymnopteridifrons*, 2n=72. 14. *Thelypteris aurita*, 2n=c.178. 15. *Woodwardia radicans*, 2n=68. 16. *Deparia japonica*, 2n=c.240. 17. *Lepisorus scolopendrium*, 2n=100. Scale bar = 10 μm.

6. *Adiantum philippense*

*Adiantum* is a large, diverse genus of about 200 species, widespread in tropical and subtropical regions. About 30 Chinese species distribute in warmer region and Southwest China (Wu and Ching 1991); a few species of this genus were cytologically studied. The base number was reported as x=29, 30, and most species observed in China were sexual diploids, except *A. diaphanum* Bl. with sexual tetraploid and agamosporous triploid and a sexual hexaploid in *A. bonatianum* Brause (Chiu 1981, Wang and Sun 1982, Wang et al. 1984, Weng 1990, Kato et al. 1992). *Adiantum philippense* is a widespread species, distributed throughout the Old World tropics, extending to Central and South America. Cytologically, it is a very variable species which was reported as n=30 (Mahabale and Kamble 1981, Raj and Manickam 1987); n=90 (Ghatak 1977) and n=90, 2n=90 (Roy et al. 1971) from outside China.

Based on our Chinese specimen, it is newly observed that the somatic chromosome number is 2n=60 (Fig. 9), and the spore number in each sporangium is counted as subnormal 32 and irregular 16. These are very peculiar figures in determining the reproductive manner. As all the sexual species ever observed in the genus had 64-spores in each sporangium, it is hardly believable that *A. philippense* has 32-spored sexual reproduction as in the case of Lindsaeaceae (Lin et al. 1990). It is suggested as an agamosporous diploid, although further cytological study is pending.

7. *Gymnopteris vestita*

The genus *Gymnopteris* consists of about six to seven species distributed in South America and South Himalaya and five Chinese species in Southwest and Northwest. It is an ally of the genus *Hemionitis*, and x=30 was recognized as the basic number of this group. Chromosome number of *G. vestita* was reported as n=30 from W. Himalaya (Verma and Golonath 1967). A number of 2n=58 (2x) is counted here (Fig. 10), and the basic number is suggested to be x=29 but not 30. It is indicated that an aneuploidy exists in this species.

8. *Lepisorus scolopendrium*

*Lepisorus* consists of about 50 species widely distributed in tropic to temperate regions of Asia, mainly in China and North India. Also on their chromosome numbers, a variety of basic numbers has been reported in this genus (n=25, 26, 35, 50, 52, 70, 75, and 2n=50, 52, 70, 72, 74, 94, 95, 100, 102, 140, 148, 150 etc.; Mitui 1965, 1966, 1968, 1970, 1971, 1973, Takei 1974, 1978, Wang et al. 1984, Masuyama et al. 1987). Polyploidy, aneuploidy, and polybasic chromosome numbers seem to show important aspects of the evolution of this genus. Only one species has ever been observed in China cytologically, and it is *L. coawlaneus* (n=70, sexual tetraploid) from Mt. Emei, Sichuang (Wang et al. 1987).

*Lepisorus scolopendrium*, which had been treated as a variety of *L. excavatus* (Bory) Ching by some authors, was determined here to have 2n=100 (Fig. 17), sexual tetraploid. The chromosome number of *L. excavatus* was reported as n=35 by Khullar et Mhara (1972). Our cytological observation supports a view that *L. scolopendrium* is an independent species from *L. excavatus*.

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林 蘇娟，岩槻邦男，加藤雅啓: **中国雲南省産シダ類の細胞分類学的研究 I.**

1990年に中国雲南省の西双版納及び大理で採集したシダ植物の胞子を培養することにより胞子体を得た．その根端の体細胞分裂と証拠標本の胞子囊あたりの胞子数を観察し，18種の染色体数と生殖様式を明らかにした．特筆すべき新知見は以下のようである．イワヒメワラビの染色体の基本数は，これまでx=49, 52, 98が報告されていたが，本研究では2n=116（X=29）であった．また中国西南部からインドにかけて分布する*Gymnopteris vestita*ではx=30が報告されていたが，今回は2n=58（x=29）であった．*Adiantum philippense*の染色体数は中国産のものは未発表であったが，本研究により2n=60の2倍体であることが明らかになった．その生殖様式については胞子囊あたりの胞子数が32個のものが無配生殖であることが示唆された．東アジア熱帯アジアに広く分布するシケシダとナチシケシダでは様々な倍数体の報告があるが，雲南省産のものは共に2n=c.240の6倍体で，日本産のものと同じであった．***Lepisorus excavatus***と***L. scolopendrium***は同種であるとする考えもあるが，本研究で観察された*L. scolopendrium*の染色体数は2n=100で，Khullar and Mehra（1972）が*L. excavatus*から報告しているn=35とは大きく異なる．このことは，この2分類群が別種であることを支持すると考えられる．

（[a]立教大学理学部，[b]東京大学大学院理学系研究科生物科学）